

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:)	Confirmation No.: 9907
)	
Etsuro OGATA et al)	Art Unit: 1643
)	
I.A. Filing Date: 08/20/1999)	Examiner: Alana M. Harris
371(c) Date: February 21, 2001)	
)	ATTY.'S DOCKET: OGATA=4
U.S. Appln. No.: 09/763,370)	
)	
For: METHOD OF DIAGNOSING BONE)	January 31, 2008
METASTASIS...)	

COMMUNICATION: SUBMISSION OF DECLARATION AND ATTACHMENTS

Honorable Commissioner for Patents
U.S. Patent and Trademark Office
Randolph Building, **Mail Stop: Amendment**
401 Dulany Street
Alexandria, VA 22314

Sir:

In applicant's RCE filed October 31, 2007, applicants requested (and were granted) a suspension term of three (3) months in which to submit further documentation, including possibly further amendments, declarations, arguments, etc.

Applicants now avail themselves of such three month suspension by attaching hereto a Declaration in the name of Tetsuya Aratani, the purposes of which are (1) to provide factual support for the amendment which has been erroneously held to constitute prohibited "new matter", and also (2) to explain how the person skilled in the art (the person addressed under Section 112) would understand the invention, as claimed, from the original description (specification).

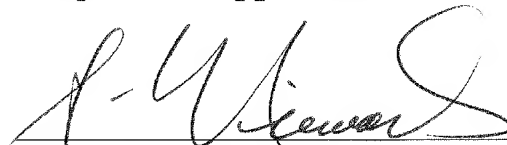
The attached Declaration speaks for itself, so undersigned (on behalf of applicants) will add no further remarks at the present time. Applicants only note for the record, respectfully, that a Declaration is **evidence**, and must be accepted by the PTO in the absence of evidence to the contrary.

Applicants again respectfully request favorable reconsideration and allowance.

Respectfully submitted,

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application:

E. OGATA et al

Application No. 09/763,370

Filed: February 21, 2001

For: METHOD OF DIAGNOSING BONE METASTASIS OF MALIGNANT TUMOR

DECLARATION UNDER 37 CFR 1.132

Honorable Commissioner for Patents

U.S. Patent and Trademark Office

Customer Service Window

Randolph Building, Mail Stop Amendment

401 Dulany Street

Alexandria, VA 22314

Sir:

I, Tetsuya Aratani, a Japanese citizen, hereby declare and state that I am familiar with the subject matter contained therein.

I declare that I received a Bachelor of Pharmaceutical Sciences from Kyoto University of Pharmaceutical Sciences in March 1986.

I also declare that I have been employed by Chugai Pharmaceutical Co. Ltd., the Assignee of this application, since April 1986, and that I now work as a Patent Group Manager for the Intellectual Property Department.

I declare further that the following discussion about the rejections under 35 U.S.C. §112 is true and correct to the best of my knowledge.

DISCUSSION ABOUT THE REJECTIONS UNDER 35 U.S.C. §112 RAISED IN

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THE OFFICE ACTION MAILED JULY 27, 2007

The rejections under 35 U.S.C. §112 raised in the Office Action mailed July 27, 2007 are discussed below.

1. New matter rejection in section 6 of the Office Action

a. Applicants corrected the mistranslation of the definition of the Z value during the prosecution of the corresponding European application. We are attaching a copy of the reply to the Communication pursuant to Article 96(2) EPC issued by the European Patent Office on July 25, 2005. In this reply, the same arguments and explanations as in the previously filed responses during the prosecution of the present application are given. The European examiner in charge of the European application, who is a person skilled in the art, accepted this correction. We are attaching a copy of the Communication pursuant to Article 96(2) EPC issued by the European Patent Office on July 5, 2007. Reference to the sentence numbered 9 appearing on page 4 of the Communication is respectfully requested.

b. The word "layer" in the recitation "wherein a layer crossover index indicates amelioration of the patient's condition" appearing in the previous claim 8 should be corrected to "higher" and we corrected the incorrect word "layer" accordingly in REPLY TO FINAL ACTION filed on October 31, 2007. This correction is supported by the statement appearing on page 12, lines 21-26: "The results of Example 1 and 2 showed the patients with bone metastasis in Group CR who were effectively treated by drugs had high crossover index values whereas the patients with bone metastasis in Group PD who changed for the worse without any therapeutic effect had low crossover index values."

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c. The Examiner objects to "at least one of" and "osteoblasts or at least one of" in the previous claim 8. The phrase "at least one of" was deleted in REPLY TO FINAL ACTION filed on October 31, 2007. A formative marker that reflects the activity of osteoblasts and a marker that reflects the activity of osteoclasts are described in the specification. Thus, it is considered that this objection would be overcome.

2. Rejection in section 8 of the Office Action

a. This objection has close relation to that of paragraph (b) of section 6. Thus, this objection would also be overcome by the explanation given in paragraph (b) of the section numbered 1 above.

b. The Examiner states that claims 2-6, 8-17 and 25-30 continue to be vague and indefinite because it is not clear from the claims how the two markers are used in ascertaining efficacy of a drug, and that the method steps are not clear. The Examiner also objects to claim 8. However, the objection to claim 8 would be overcome by paragraphs (b) and (c) of the section numbered 1 above. In addition, if those skilled in the art read Applicants' specification, they would very well understand how the two markers are used in ascertaining efficacy of a drug. This is evidenced by the fact that the corresponding European application, European Patent Application No. 99938547.9 has been granted. This fact demonstrates that the European examiner in charge of the European application, who is a person skilled in the art, very well understand the present invention. For reference, I am attaching a copy of the Communication under Rule 51(4) EPC dated October 22, 2007.

d. This rejection would be overcome because the phrase "testing serum of said patient for a marker of bone

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formation" to which the Examiner objects is deleted in REPLY TO FINAL ACTION filed on October 31, 2007.

e. Such a Jepson-type claim as claim 32 has been accepted by the United States Patent and Trademark Office. For example, United States Patent 7,289,109; 7,289,020; 7,288,690; and 7,288,620 contain claims having such Jepson language. Thus, withdrawal of this rejection is respectfully requested.

I declare further that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Dated this 30th day of January 2008

Tetsuya Aratani
Tetsuya Aratani

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Partnerschaftsregister Amtsgericht München PR 69

EP 99 93 8547.9-2404
Etsuro Ogata
Our Ref.: F1079 EP S3

Munich, April 4, 2006
JAE/RPL/HÄ

This is in reply to the Communication pursuant to Article 96(2) EPC issued by the European Patent Office on July 25, 2005.

Enclosed please find new claims 1 to 9 which should form the basis for further substantive examination. We herewith reserve our right to file divisional applications for subject matter which has been deleted from the claims or which is no longer covered by the new set of claims.

1. AMENDMENTS TO THE CLAIMS

1.1 New claim 1 corresponds to previously filed claim 1 except for the following amendments:

- (i) The term "diagnosing bone metastasis" has been amended to read "diagnosing amelioration and/or exacerbation of bone metastasis". Support can be found on page 12, line 26 to page 13, line 1, and on page 16, lines 18 to 24 of the application as filed.
- (ii) The term "in a patient with a cancer disease" has been introduced following the term "malignant tumor". Support can be found in Examples 1 to 3 relating to measurements on cancer patients.

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- (iii) Within the term "a marker that reflects the activity of osteoblasts", the word "marker" has been amended to read in the plural form. Support can be found on page 14, lines 5 to 10 of the application as filed.
- (iv) The term "action of osteoclasts" has been amended to read "activity of osteoclasts" in order to generate consistency with the wording of the preceding term "activity of osteoblasts".
- (v) Subsection (a) has been introduced and corresponds to previously filed claim 4(a).
- (vi) Subsection (b) has been introduced and is based on the disclosure content of the application as filed. In particular, support can be found on page 7, lines 22 to 26, on page 8, lines 26 and 27, on page 13, line 19 to page 14, line 10, on page 14, line 24 to page 15, line 1, on page 16, lines 18 to 24, and in Example 1 (in particular, on page 9, lines 5 to 8, and on page 10, lines 7/8 and lines 24 to 27) of the application as filed.

1.2 New claim 2 corresponds to previously filed claim 2 except for the following amendments:

- (i) Within the term "a marker that reflects the activity of osteoblasts", the word "marker" has been amended to read in the plural form. Support can be found on page 14, lines 5 to 10 of the application as filed.
- (ii) The term "action of osteoclasts" has been amended so as to read "activity of osteoclasts" in order to generate consistency with the wording of the preceding term "activity of osteoblasts".
- (iii) Subsection (a) has been introduced and finds support in previously filed claim 4(b) as well as on page 14, lines 2 and 3 of the application as filed.
- (iv) Subsection (b) has been introduced and is based on the disclosure content of the application as filed. In particular, support can be found as indicated for subsection (b) of new claim 1; see section 1.1 (vi), supra.

1.3 New claims 3 to 9 corresponds to previously filed claims 3 to 9.

No new matter has been introduced with the amendments made to the claims.

2. NOVELTY (ARTICLE 54 EPC)

2.1 In section 2 of the Communication the Examining Division holds the view that the subject matter of previously filed claims 1 to 3 lacks novelty pursuant to Articles 52(1) and 54(1) and (2) EPC in view of D1, D2 and D4 to D6, and that the subject-matter of previously filed claims 2 and 3 lacks novelty pursuant to Articles 52(1) and 54(1) and (2) EPC in view of D3.

2.2 We cannot agree with the Examining Division's view as will be explained in the following. In this connection, please find enclosed an English translation of the original Japanese documents D1, D2, and D3, which are addressed when citing text passages out of them.

2.2.1 Novelty in view of D1 (Koizumi, 1998)

The Examining Division's novelty objection in view of D1 is apparently based on the corresponding statement in the International Search Report (should probably read: International Preliminary Examination Report). Accordingly, D1 allegedly discloses a diagnostic method for bone metastasis of malignant tumors that uses a marker that reflects the activity of osteoblasts and a marker that reflects the activity of osteoclasts.

D1 relates to bone metastasis of cancers and bone metabolic markers. In particular, it is said in the introduction of D1 that bone metastasis is diagnosed by imaging techniques such as X-rays, bone scintigraphy, computed tomography and magnetic resonance imaging. Furthermore, it is said on page 4, middle, that for the diagnosis of bone metastasis, bone resorption markers are considered to be more useful than bone formation markers, and that it may be required, however, to combine bone formation markers with bone resorption markers. Furthermore, it is said that in spite of various markers available for the evaluation of formation and resorption of bone, it is difficult to conclude at present which marker is the best. In addition, it is said at the bottom of page 4 that since the sensitivity of bone metabolic markers is not high, the diagnosis of bone metastasis cannot be relied solely on these markers, but it may be possible to use the markers as a supplementary means for the diagnosis of bone metastasis.

Accordingly, D1 does not disclose the method of diagnosing amelioration and/or exacerbation of bone metastasis of malignant tumor in a cancer patient as described in new claim 1, and D1 does not disclose the method of evaluating the therapeutic efficacy of a drug as described in new claims 2 and 3. Therefore, new claims 1 to 3 are novel in view of D1.

2.2.2 Novelty in view of D2 (Takahashi and Koizumi, 1997)

The Examining Division holds the view that D2 (also) teaches combinations of bone resorption and formation markers that increases the sensitivity.

D2 relates to the significance of bone metabolic markers for diagnosis of bone metastasis. In particular, D2 describes the major markers of bone metabolism and their significance in the diagnosis and follow-up observation of bone metastasis of cancer (see Introduction). It is said in the bottom part of the summary that the combination of resorption and formation markers increased sensitivity, and that bone metabolic markers would be useful not only to detect bone metastases but also to monitor therapeutic effect, and they could partly substitute for bone scintigraphy. Furthermore, it is said that bone metabolic markers, especially when two or three markers are combined, are useful for the diagnosis and follow-up observation of bone metastasis, and that they are to be used as markers of treatment effects that work more sensitively and rapidly than bone scintigraphy (see Conclusion).

However, D2 does not disclose the method of diagnosing amelioration and/or exacerbation of bone metastasis of malignant tumor in a cancer patient as described in new claim 1, and D2 does not disclose the method of evaluating the therapeutic efficacy of a drug as described in new claims 2 and 3. Therefore, new claims 1 to 3 are novel in view of D2.

2.2.3 Novelty in view of D3 (Nakaba et al. 1995)

The Examining Division's novelty objection in view of D3 is apparently based on the corresponding statement in the International Search Report (should probably read: International Preliminary Examination Report). Accordingly, D3 allegedly describes a means of determining the effect of drug therapy using a marker that reflects osteoblast activity and a marker that reflects osteoclast activity.

D3 relates to the correlation between evaluation of therapeutic effect on bone metastatic lesions, bone metabolic markers, and the disease state of bone metastasis. It is said in D3 that bone metastasis in patients with advanced cancer is often complicated by metastasis in other organs, and in many cases, adequate treatment is not possible due to metabolic disorders and dysfunction of other important organs (see Introduction). The therapeutic effect was rated according to the degree of bone reconstitution in metastatic lesions (1st paragraph of "Results"). The study described in D3 (i.e., three case reports) showed that when chemotherapy or radiotherapy for metastatic lesions of cancer is so effective that bone formation is achieved, the therapeutic effect is considered to be "good" or better according to the evaluation criteria of the Japan Society of Clinical Oncology, and recurrence rate is low in the short term (see Discussion, 2nd paragraph).

However, D3 does not disclose the method of evaluating the therapeutic efficacy of a drug as described in new claims 2 and 3. Therefore, new claims 2 and 3 are novel in view of D3.

2.2.4 Novelty in view of D4 (Christenson, 1997)

The Examining Division holds the view that D4 (also) teaches combinations of bone resorption and formation markers that increases the sensitivity.

D4 presents an overview of biochemical markers of bone metabolism along with indications for their clinical utilization. D4 mainly refers to the principles of bone metabolism with a subsection focussing on markers of bone metabolism; starting on page 528, corresponding clinical studies involving markers of bone metabolism are referred to.

In particular, on page 585, D4 relates to bone markers in connection with patients having malignancies, wherein differences in the expression rate of bone markers between cancer patients with and without bone metastasis were monitored. However, it is said on page 585, right column, 3rd paragraph, that the number and variety of cancer patients that have been examined is clearly inadequate for reaching a definitive conclusion regarding the utilization of bone markers for monitoring patients with malignancies. In addition, it is said that there clearly is need for other well-designed studies that additionally address the association of bone markers and malignancies. It is concluded on page 588, right column, 3rd paragraph, that biochemical markers of bone formation and resorption provide a new and potentially important tool for the assessment and monitoring of bone metabolism. It is noted that the characteristics of any marker are largely a function of the assay used for the assessment of the marker. Furthermore, it is said that continued efforts aimed at improving analysis and interpretation of markers must continue, and that fundamental issues remain to be fully elucidated.

In summary, when referring to bone markers, D4 described the monitoring of patients with malignancies using bone markers. However, D4 does not disclose the method of diagnosing amelioration and/or exacerbation of bone metastasis of malignant tumor in a cancer patient as described in new claim 1, and D4 does not disclose the method of evaluating the therapeutic efficacy of a drug as described in new claims 2 and 3. Therefore, new claims 1 to 3 are novel in view of D4.

2.2.5 Novelty in view of D5 (Plebani et al., 1996)

The Examining Division holds the view that D5 (also) teaches combinations of bone resorption and formation markers that increases the sensitivity.

D5 relates to new and traditional serum markers of bone metabolism in the detection of skeletal metastases. In particular, the sensitivity, specificity, and efficiency of "new" and "traditional" biochemical serum markers of osteoblastic and osteoclastic activity in the detection of bone metastases were compared (page 68, left column, 3rd paragraph). In particular, in order to assess traditional markers, ALP, TrACP and BGP were measured; in order to assess new markers, ALP-B, PICP, and ICTP were measured (page 68, left

column, 6th and 7th paragraph). It is said that a better understanding of the separate roles of bone formation and bone resorption would clarify some aspects of metabolic skeletal abnormalities in disease states, allowing the diagnosis to be established and the influence of therapy on bone formation and resorption to be monitored (page 70, left column, 2nd paragraph). The authors of D5 have considered different markers of bone synthesis and degradation and have compared the clinical performance of "traditional" and "new" markers of bone turnover in the assessment of metastatic changes in bone metabolism (page 70, left column, 5th paragraph). Furthermore, it is said that all markers, except for PICP, give serum values that are significantly higher in patients with bone metastasis than in age-matched controls (page 70, paragraph bridging left and right column). In the last sentence it is said that after malignancy has been diagnosed, determination of bone ALP activity and ICTP concentration may provide a non-invasive test for monitoring patients both with osteoblastic metastases and osteolytic metastases.

Accordingly, D5 does not disclose the method of diagnosing amelioration and/or exacerbation of bone metastasis of malignant tumor in a cancer patient as described in new claim 1, and D5 does not disclose the method of evaluating the therapeutic efficacy of a drug as described in new claims 2 and 3. Therefore, new claims 1 to 3 are novel in view of D5.

2.2.6 Novelty in view of D6 (Meijer et al., 1998)

The Examining Division holds the view that D6 (also) teaches combinations of bone resorption and formation markers that increases the sensitivity.

D6 relates to biochemical parameters of bone metabolism in bone metastases (BMS) of solid tumors. In particular, the role of biochemical markers of bone metabolism in the diagnosis and monitoring of BMS in solid tumors is reviewed. It is said that the markers referred to in D6 can often identify BMS before visualization by imaging techniques, however, the precise role of said markers of bone metabolism in early diagnosis and monitoring of BMS needs further evaluation in longitudinal studies (see middle and bottom of abstract). In section 3 (i.e., page 9 ff.), the clinical application of biochemical markers of bone metabolism in various patient groups is discussed. However, it is said

that a comparison between patient studies is difficult due to a difference in the definition of bone metastases since in some studies BMS were detected by bone scintigraphy, whereas in others the presence of BMS was confirmed by plain radiography, which might lead to an inequality in the degree of bone involvement (page 9, right column, 3rd paragraph). In section 4 (discussion) it is mentioned that biochemical markers of bone metabolism may indicate bone metastases before metastases become visible by imaging means (page 17, left column, 3rd paragraph). Furthermore, in section 5 (conclusion) it is said that formation markers as well as resorption markers may indicate early bone metastases (page 18, left column, 2nd paragraph).

Accordingly, D6 does not disclose the method of diagnosing amelioration and/or exacerbation of bone metastasis of malignant tumor in a cancer patient as described in new claim 1, and D6 does not disclose the method of evaluating the therapeutic efficacy of a drug as described in new claims 2 and 3. Therefore, new claims 1 to 3 are novel over the disclosure content of D6.

- 2.3 In conclusion, none of D1 to D6 discloses the methods described in new claims 1 to 3, in particular the combination of the characteristic features of new claims 1 to 3. Accordingly, it is respectfully submitted that new claims 1 to 3 fulfil the requirements of Articles 52(1) and 54(1) and (2) EPC in view of any one of D1 to D6. Therefore, the Examining Division's objection is overcome.

3. INVENTIVE STEP (ARTICLE 56 EPC)

- 3.1 In section 3 of the Communication the Examining Division holds the view that previously filed claims 4 to 9 lack inventive step pursuant to Articles 52(1) and 56 EPC in view of D2 to D6. In particular, the Examining Division holds the view that previously filed claims 4 to 9 differ from D3 in that specific combinations of markers are used for determining the effect of a drug therapy, and that the problem to be solved by claims 4 to 9 may therefore be regarded as how to improve the determination of the effect of a drug therapy. Accordingly, the Examining Division alleges that the solution proposed in previously filed claims 4 to 9 cannot be considered as involving an inventive step because the skilled person would have had an incentive to use the specific combinations

of markers disclosed in D4 to D6, in particular in view of the last sentence of the abstract of D2.

3.2 We cannot agree with the Examining Division's objection. This will be explained in the following.

3.2.1 The disclosure content of D3 was referred to when discussing novelty of claims 2 and 3 in view of D3 (see section 2.2.3, supra). It was shown that D3 does not disclose the method of evaluating the therapeutic efficacy of a drug as described in new claims 2 and 3. Moreover, it is submitted that D3 does not at all describe a means of determining the effect of drug therapy using a marker that reflects osteoblast activity and a marker that reflects osteoclast activity. However, this was alleged in the IPER, and in the following picked up by the Examiner as a basis for the "problem-and-solution" approach described in section 3 of the Communication. Accordingly, it is submitted that the "problem-and-solution" approach based on D3 in combination with D2 and D4 to D6 is not justified since it appears to be based on an incorrect statement in the IPER concerning the disclosure content of D3.

3.2.2 However, new claims 1 to 9 are inventive over the cited prior art documents. In particular, new claims 1 to 9 are characterized not only by the specific combinations of bone metabolic markers, but also by diagnosing bone metastasis of malignant tumor or evaluating the therapeutic efficacy of a drug using a "Z value" which is calculated based on the combination of specific bone metabolic markers and values obtainable by the measurement thereof. None of D1 to D6 suggest or teach the invention of new claims 1 to 9, nor does any combination of any of D1 to D6. Thus, it would have been difficult for the person skilled in the art to arrive at the invention of new claims 1 to 9, even if the skilled person had combined any of D1 to D6. Therefore, it would not have been obvious for the person skilled in the art to arrive at the invention provided by the present application.

Accordingly, it is respectfully submitted that new claims 1 to 9 fulfil the requirements of Articles 52(1) and 56 EPC in view of the disclosure content of any one of D1 to D6.

Therefore, a favourable reconsideration of the subject-matter is kindly requested.

4. CORRECTION UNDER RULE 88 EPC

4.1 We herewith request the correction of page 9 of the English translation of the description pursuant to Rule 88 EPC. Please find enclosed the correspondingly amended page 9.

4.2 In particular, it is requested that the term "with bone metastasis" spanning lines 6 and 7 on page 9 be corrected to read "without bone metastasis". An amendment pursuant to Rule 88 EPC is requested. In this connection it is respectfully submitted that the term "with bone metastasis" is a translational error. Said term is an English translation of the corresponding Japanese term on page 6, line 23 of the corresponding Japanese text of PCT/JP99/04480. Please find enclosed page 6 of the Japanese text of PCT/JP99/04480 indicating the relevant term. However, this Japanese term should have been translated as "without bone metastasis", not "with bone metastasis". This is furthermore supported by the fact that said Japanese term also appears a second time in line 24 of page 6 of the Japanese text of PCT/JP99/04480. This second term has been translated correctly as can be derived from page 9, lines 7/8 of the English translation of the description. Accordingly, the person skilled in the art can clearly and unambiguously derive that the term "with bone metastasis" spanning lines 6 and 7 on page 9 of the English translation of the description should read "without bone metastasis".

It is respectfully submitted that the requested correction complies with the requirements of Rule 88 EPC and Article 123(2) EPC since new no matter is added.

5. REQUESTS

With the above explanations and the proposed modification of the claims, the Applicant has met the requirements set forth in the Communication. The adaptation of the description is requested to be postponed until a final agreement on the wording of the claims has been reached.

If, however, the Examining Division does not agree with the above, it is requested that either a further Communication pursuant to Art. 96(2) EPC or a summons to attend oral proceedings according to Art. 116(1) EPC be issued. If deemed expedient, an informal interview is requested. The undersigned is prepared to discuss minor amendments over the phone.



Dr. Hans-Rainer Jaenichen
European Patent Attorney

Encl.:

New claims 1 to 9;

Full English translation of D1, D2 and D3;

Page 6 of the original Japanese text of PCT/JP99/04480

indicating the relevant term for correction pursuant to Rule 88 EPC;

Amended page 9 of the English translation of PCT/JP99/04480

with handwritten amendment and as clean copy thereof

EP 99 93 8547.9
Etsuro Ogata
Our Ref.: F1079 EP S3

New set of claims

1. An in vitro method of diagnosing amelioration and/or exacerbation of bone metastasis of malignant tumor in a patient with a cancer disease using markers that reflect the activity of osteoblasts and a marker that reflects the activity of osteoclasts,
 - (a) wherein the markers that reflect the activity of osteoblasts are
 - (i) a marker associated with the phase of calcification; and
 - (ii) a marker associated with the phase of osteoblast proliferation and/or matrix formation;
 - (b) wherein the marker that reflects the activity of osteoclasts is a marker associated with osteoclasts targeted to evaluation of worsening of the disease, comprising testing a blood sample for a marker of bone metabolism, wherein the amelioration of bone metastasis and the degree of the exacerbation of bone metastasis are diagnosed by monitoring said markers, which method is characterized in that said testing comprises measuring for both osteocalcin and one marker selected from BALP, PICP and PINP, determining a Z value for each of said osteocalcin and said marker, each Z value being determined by dividing the difference between said measured value for said patient and an average value for patients without bone metastasis, by a standard deviation of a patient without bone metastasis, and determining a crossover index by dividing said Z value for osteocalcin by said Z value for BALP, PICP or PINP, said crossover index providing a diagnosis of progression of bone metastasis in the treatment of said patient for said cancer.
2. An in vitro method of evaluating the therapeutic efficacy of a drug using markers that reflect the activity of osteoblasts and a marker that reflects the activity of osteoclasts,
 - (a) wherein the markers that reflect the activity of osteoblasts are

- (i) a marker associated with the phase of calcification; and
 - (ii) a marker associated with the phase of osteoblast proliferation and/or matrix formation;
 - (b) wherein the marker that reflects the activity of osteoclasts is a marker associated with osteoclasts targeted to evaluation of worsening of the disease, comprising testing a blood sample for a marker of bone metabolism, wherein the amelioration of bone metastasis or therapeutic effect and the degree of the exacerbation of bone metastasis are diagnosed correctly by monitoring said markers, which method is characterized in that said testing comprises measuring for both osteocalcin and one marker selected from BALP, PICP and PINP, determining a Z value for each of said osteocalcin and said BALP, each said Z value being determined by dividing the difference between said measured value for a patient and an average value for patients without bone metastasis, by a standard deviation of a patient without bone metastasis, and determining a crossover index by dividing said Z value for osteocalcin by said Z value for BALP, PICP or PINP, said crossover index providing a diagnosis of progression of bone metastasis and evaluation of drug efficacy in the treatment of said patient.
3. The method according to claim 2, wherein the drug is a cancer control therapeutic agent, a bone resorption suppressant or an endocrine therapeutic agent.
4. The method according to any one of claims 1 to 3, wherein the marker that reflects the activity of osteoblasts is:
- (a) a marker associated with the phase of osteoblast proliferation and matrix formation and a marker associated with the phase of calcification; or
 - (b) a marker associated with the phase of matrix maturation and a marker associated with the phase of calcification.
5. The method according to any one of claims 1 to 4, wherein the marker that reflects the activity of osteoblasts is:
- (a) PICP or PINP and osteocalcin; or

- (i) a marker associated with the phase of calcification; and
 - (ii) a marker associated with the phase of osteoblast proliferation and/or matrix formation;
 - (b) wherein the marker that reflects the activity of osteoclasts is a marker associated with osteoclasts targeted to evaluation of worsening of the disease, comprising testing a blood sample for a marker of bone metabolism, wherein the amelioration of bone metastasis or therapeutic effect and the degree of the exacerbation of bone metastasis are diagnosed correctly by monitoring said markers, which method is characterized in that said testing comprises measuring for both osteocalcin and one marker selected from BALP, PICP and PINP, determining a Z value for each of said osteocalcin and said BALP, each said Z value being determined by dividing the difference between said measured value for a patient and an average value for patients without bone metastasis, by a standard deviation of a patient without bone metastasis, and determining a crossover index by dividing said Z value for osteocalcin by said Z value for BALP, PICP or PINP, said crossover index providing a diagnosis of progression of bone metastasis and evaluation of drug efficacy in the treatment of said patient.
3. The method according to claim 2, wherein the drug is a cancer control therapeutic agent, a bone resorption suppressant or an endocrine therapeutic agent.
4. The method according to any one of claims 1 to 3, wherein the marker that reflects the activity of osteoblasts is:
- (a) a marker associated with the phase of osteoblast proliferation and matrix formation and a marker associated with the phase of calcification; or
 - (b) a marker associated with the phase of matrix maturation and a marker associated with the phase of calcification.
5. The method according to any one of claims 1 to 4, wherein the marker that reflects the activity of osteoblasts is:
- (a) PICP or PINP and osteocalcin; or

(b) BALP and osteocalcin.

6. The method according to any one of claims 1 to 5, wherein the marker that reflects the action of osteoclasts is a marker associated with bone type I collagen.
7. The method according to any one of claims 1 to 6, wherein the marker that reflects the action of osteoclasts is deoxypyridinoline and/or ICTP.
8. The method according to any one of claims 1 to 7, which is based on the value of a crossover index or the ratio between a marker associated with the phase of calcification and a marker associated with the phase of osteoblast proliferation and matrix formation and the measured value of a marker associated with bone type I collagen, or on the value of crossover index or the ratio between a marker associated with the phase of calcification and a marker associated with the phase of matrix maturation and the measured value of a marker associated with bone type I collagen.
9. The method according to claim 8, which is based on the value of a crossover index between osteocalcin and PICP or PINP and the measured value of ICTP, or on the value of a crossover index between osteocalcin and BALP and the measured value of ICTP.

EP 99 93 8547.9-2404

Etsuro Ogata

Our Ref.: F1079 EP S3

以下の2つの実施例によって確認した。

実施例 1

1994年10月から1996年4月の間に、骨転移を有する前立腺癌患者4
3名、骨転移なしの前立腺癌患者46名を対象として、骨形成マーカーのレベル
を調べた。明らかに骨転移なしの前立腺癌患者46名のうち、29名は前立腺切
除または放射線治療を受けた経験があり、その他の17名は新たに診断を受けた
患者であり、骨シンチと血清サンプリングの後で前立腺切除または放射線治療を
受けた。骨転移なしの患者の平均年齢は69歳（年齢範囲47歳－85歳）であ
った。これらの患者の癌の進行度合は、4名の患者がステージA、14名の患者
がステージB、19名の患者がステージC、9名の患者がステージD1であった。
一方、骨転移を有する患者43名のうち、9名は新たに診断を受けて、骨シンチ
と血清サンプリングの後でホルモン療法を受けていた。その他の34名の患者は
ホルモン療法および／または化学療法による積極的な治療をこれらの療法の開始
時から種々の間隔で受けていた。骨転移を有する患者の平均年齢は69歳（年齢
範囲53歳－83歳）であった。

すべての患者に関して、インフォームドコンセントが得られた後、骨シンチの
際に採血して血清を分離し、分析を行うまで－40℃で冷凍保存した。血清中の
BALPをアルクファーズBキット（ALKPHASE-B kit, Metra Biosystems）を
用いたエンザイムイムノアッセイ（免疫測定法）で測定した。また、血清中のオ
ステオカルシンは三菱 BGP-IRMA キット（三菱化学）を用いたイムノラジオメト
リックアッセイ（放射免疫測定法）で測定した。

結果を図2に示す。図2において、Z値とは（測定値－骨転移がない患者の平
均値）／（骨転移がない患者の標準偏差値）で定義される値である。また、図2
においてCR、flare、NC、IMP、new、PDはそれぞれ下記の意味
を有する。

・CR: complete remission（完全寛解）。

・flare: flare up（実際には治療が有効であったにもかかわらず、

EP 99 93 8547.9-2404

Etsuro Ogata

Our Ref.: F1079 EP S3

Serum BALP was measured by enzyme immunoassay with an ALKPHASE-B kit of Metra Biosystems. Serum osteocalcin was measured by immunoradiometric assay with a BGP-IRMA kit of Mitsubishi Chemical Corp.

5 The results are shown in Fig. 2, in which Z value is defined by (measured value - average for the patients ^{without} ~~with~~ bone metastasis)/(standard deviation of a patient without bone metastasis). In Fig. 2, CR, flare, NC, IMP, new and PD have the following respective meanings.

10 CR : complete remission

flare : flare-up [the treatment was effective but the bone metastasis appeared to have progressed on a bone scan (scintigraphic) image].

NC : no change (no change was observed).

15 IMP : improvement (a sign of improvement was recognized).

new : diagnosed to have a new bone metastasis.

PD : progression of disease (the disease was found to have progressed).

20 For each of these patient groups, BALP and osteocalcin had the following Z values.

Z value of BALP

CR : 2.18

flare : 3.40

NC : 8.23

25 IMP : 2.39

new : 1.82

PD : 24.50

Z value of osteocalcin

Serum BALP was measured by enzyme immunoassay with an ALKPHASE-B kit of Metra Biosystems. Serum osteocalcin was measured by immunoradiometric assay with a BGP-IRMA kit of Mitsubishi Chemical Corp.

5 The results are shown in Fig. 2, in which Z value is defined by (measured value - average for the patients without bone metastasis)/(standard deviation of a patient without bone metastasis). In Fig. 2, CR, flare, NC, IMP, new and PD have the following respective meanings.

- 10 CR : complete remission
flare : flare-up [the treatment was effective but the bone metastasis appeared to have progressed on a bone scan (scintigraphic) image].
NC : no change (no change was observed).
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new : diagnosed to have a new bone metastasis.
PD : progression of disease (the disease was found to have progressed).

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flare : 3.40
NC : 8.23
25 IMP : 2.39
new : 1.82
PD : 24.50

Z value of osteocalcin



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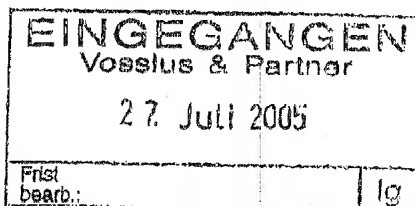
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Direction Générale 2

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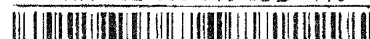


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RK 52 038 716 8DE 110



Application No. 99 938 547.9 - 2404	Ref. F 1079 EP	Date 25.07.2005
Applicant Ogata, Etsuro		

Communication pursuant to Article 96(2) EPC

The examination of the above-identified application has revealed that it does not meet the requirements of the European Patent Convention for the reasons enclosed herewith. If the deficiencies indicated are not rectified the application may be refused pursuant to Article 97(1) EPC.

You are invited to file your observations and insofar as the deficiencies are such as to be rectifiable, to correct the indicated deficiencies within a period

of 4 months

from the notification of this communication, this period being computed in accordance with Rules 78(2) and 83(2) and (4) EPC.

One set of amendments to the description, claims and drawings is to be filed within the said period on separate sheets (Rule 36(1) EPC).

Failure to comply with this invitation in due time will result in the application being deemed to be withdrawn (Article 96(3) EPC).



Stricker, J-E
 Primary Examiner
 for the Examining Division

Enclosure(s): 3 page/s reasons (Form 2906)
 XP001027215



Bescheld/Protol .Anlage)

Communication/Minutes (Annex)

Notification/Procès-verbal (Annexe)

Datum
Date 25.07.2005
DateBlatt
Sheet 1
FeuilleAnmelde-Nr.:
Application No.: 99 938 547.9
Demande n°:

The examination is being carried out on the following application documents:

Description, Pages

1-17 filed with entry into the regional phase before the EPO

Claims, Numbers

1-9 filed with entry into the regional phase before the EPO

Drawings, Sheets

1/4-4/4 filed with entry into the regional phase before the EPO

The following documents are referred to in this communication; the numbering will be adhered to in the rest of the procedure:

- D1: M. KOIZUMI: 'Bone metastasis of cancer and osteal metabolism marker' CLINICAL CALSIUM May 1998, pages 98 - 100, XP002922594
- D2: TAKAHASHI S ET AL: "SIGNIFICANCE OF OSTEAL METABOLISM MARKER FOR DIAGNOSING BONE METASTASIS" BIOTHERAPY, KLUWER ACADEMIC PUBLISHERS, DORDRECHT, NL, vol. 11, no. 1, 1997, pages 75-80, XP002922593, ISSN: 0921-299X
- D3: K. NAKABA: 'Correlation among the measurement of therapeutic effects on bone metastasis foci, osteal metabolism marker and the condition of bone metastasis' THERAPEUTIC RESEARCH vol. 16, no. 12, 1995, pages 212 - 217, XP002922592
- D4: CHRISTENSON R H: "BIOCHEMICAL MARKERS OF BONE METABOLISM: AN OVERVIEW" CLINICAL BIOCHEMISTRY, PERGAMON PRESS, XX, vol. 30, no. 8, December 1997 (1997-12), pages 573-593, XP000938263, ISSN: 0009-9120
- D5: PLEBANI M ET AL: "New and traditional serum markers of bone metabolism in the detection of



Beschold/Protol	Anlage)	Communication/Minutes (Annex)	Notification/Procès-verbal (Annexe)
Datum Date Date	25.07.2005	Blatt Sheet Feuille	Anmelde-Nr.: Application No.: 99 938 547.9 Demande n°:

skeletal metastases." CLINICAL BIOCHEMISTRY, vol. 29, no. 1, 1996, pages 67-72,
XP002184395, ISSN: 0009-9120

D6: MEIJER W G ET AL: "Biochemical parameters of bone metabolism in bone metastases of solid tumors (Review)". ONCOLOGY REPORTS, Vol. 5, No. 1, January 1998, pages 5-21,
XP001027215, ISSN: 1021-335X

D6 is the full text of the database abstract cited in the ISR. A copy of that document is appended hereto.

1. The amendments to the claims, filed with entry into the regional phase before the EPO, have their basis in the originally filed application, and therefore satisfy Art. 123(2) EPC.
2. The present application does not meet the requirements of Article 52(1) EPC, because the subject-matter of claims 1-3 is not new in the sense of Article 54(1) and (2) EPC.

According to the ISR, D1 discloses a diagnostic method for bone metastasis of malignant tumors that uses a marker that reflects the activity of osteoblasts and a marker that reflects the activity of osteoclasts.

D2 and D4-D6 also teaches combinations of bone resorption and formation markers that increases the sensitivity.

D1, D2 and D4-D6 are therefore prejudicial to the novelty of claim 1.

According to the ISR, D3 describes a means of determining the effect of drug therapy using a marker that reflects osteoblast activity and a marker that reflects osteoclast activity. D3 is therefore prejudicial to the novelty of claims 2 and 3.

3. Claims 4-9 differ from D3 in that specific combinations of markers are used for determining the effect of a drug therapy. The problem to be solved by claims 4-9 may therefore be regarded as how to improve the determination of the effect of a drug therapy.



Bescheld/Proto (Anlage)

Communication/Minutes (Annex)

Notification/Procès-verbal (Annexe)

Datum
Date
Date 25.07.2005Blatt
Sheet
Foil 3Anmelde-Nr.:
Application No.: 99 938 547.9
Demande n°:

The solution proposed in claims 4-9 of the present application cannot be considered as involving an inventive step (Articles 52(1) and 56 EPC) because the skilled person would have had an incentive to use the specific combinations of markers disclosed in D4-D6, in particular in view of the last sentence of the abstract of D2.

4. Finally it is pointed out that all amendments must be carried out under strictest observation of Article 123(2) EPC. In order to ascertain whether the new claims meet the requirements of Article 123(2), the applicant is requested to indicate, in his letter of reply, where a basis may be found, in the originally filed application, for each and every amendment made to the application. The applicant is asked to furnish with respect to every amendment an additional copy showing the hand corrected original sheet (see the Guidelines E-II, 1).



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06. Juli 2007

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 (Formalities and other matters)



Application No. 99 938 547.9 - 2404	Ref. F 1079 EP	Date 05.07.2007
Applicant Ogata, Etsuro		

Communication pursuant to Article 96(2) EPC

The examination of the above-identified application has revealed that it does not meet the requirements of the European Patent Convention for the reasons enclosed herewith. If the deficiencies indicated are not rectified the application may be refused pursuant to Article 97(1) EPC.

You are invited to file your observations and insofar as the deficiencies are such as to be rectifiable, to correct the indicated deficiencies within a period

of 2 months

from the notification of this communication, this period being computed in accordance with Rules 78(2) and 83(2) and (4) EPC.

One set of amendments to the description, claims and drawings is to be filed within the said period on separate sheets (Rule 36(1) EPC).

Failure to comply with this invitation in due time will result in the application being deemed to be withdrawn (Article 96(3) EPC).



STRICKER, J
 Primary Examiner
 for the Examining Division

Enclosure(s): 4 page/s reasons (Form 2906)



Bescheid/Protokoll (Anlage)

Communication/Minutes (Annex)

Notification/Procès-verbal (Annexe)

Datum
Date 05.07.2007
DateBlatt
Sheet 1
FolioAnmelde-Nr.:
Application No.: 99 938 547.9
Demande n°:

The examination is being carried out on the following application documents:

Description, Pages

1-17 filled with entry into the regional phase before the EPO

Claims, Numbers

1-9 received on 04.04.2006 with letter of 04.04.2006

Drawings, Sheets

1/4-4/4 filled with entry into the regional phase before the EPO

1. The present set of claims does not meet the requirements of **Art. 123(2) EPC** for at least the following reasons:

- no basis could be found for "or" in item (II) of claims 1 and 2
- as indicated in the Applicant's letter, the wording of item (b) in claims 1 and 2 is a combination of different passages of the application as originally filed. Said combination is however not disclosed as such.

2. Although the Applicant has indicated that part (b) of previous claim 4 was included in **present claim 2**, in fact part (a) thereof is present.

Claim 2 is not clear (Art. 84 EPC) as part (b) deals with metastases whereas the preamble of the claim, which derives from claim 8 as originally filed, does not specify any disease.



Beschuld/Protokoll (Anlage)

Communication/Minutes (Annex)

Notification/Procès-verbal (Annexe)

Datum
Date 05.07.2007
DateBlatt
Sheet 2
FeuilleAnmelde-Nr.:
Application No.: 99 938 547.9
Demande n°:

Claim 2 is furthermore not supported by the description as required by Article 84 EPC, as its scope is broader than justified by the description and drawings. The reason therefor is that the whole application is directed primarily to the follow up of bone metastasis accompanying malignant tumors (see e.g. p.1, l.4-8).

The **first two lines of Item (b) in claims 1 and 2** do not meet the requirements of Art. 84 EPC as they attempt to define the subject-matter in terms of the result to be achieved.

Present claims 4 and 5 would appear to be superfluous and therefore do not meet the requirements of Art. 84 EPC.

3. **Claims 1 and 2** have been drafted as separate independent claims.

Under Article 84 in combination with Rule 29(2) EPC an application may contain more than one independent claim in a particular category only if the subject matter claimed falls within one or more of the exceptional situations set out in paragraphs (a), (b) or (c) of Rule 29(2) EPC.

This is not the case in the present application however, as claims 1 and 2 clearly contain overlapping scope (according to the present application, evaluating the therapeutic efficacy of a drug is the same as evaluating the degree of amelioration of a disease, see p.12, l.28 to p.12, l.1 and p.16, l.19).

In the further prosecution of the application, failure to file an amended set of claims which complies with Rule 29(2) EPC, or to submit convincing arguments as to why the current set of claims does in fact comply with these provisions, may lead to refusal of the application under Article 97(1) EPC.

4. **Novelty and Inventiveness** could be acknowledged for the use of osteocalcin in combination with BALP, PICP or PINP, together with a marker that reflects the activity of osteoclasts, for diagnosing amelioration and/or exacerbation of bone metastasis of malignant tumor in a patient with a cancer disease (in accordance with the arguments provided in the Applicant's letter dated 04.04.2006).



Bescheld/Protokoll (Anlage)

Communication/Minutes (Annex)

Notification/Procès-verbal (Annexe)

Datum
Date 05.07.2007
DateBlatt
Sheet 3
FeuilleAnmelde-Nr.:
Application No.: 99 938 547.9
Demande n°:

5. **The document cited under Ref. (44) in D4** is regarded as being the closest prior art to the subject-matter of **claim 2**, and discloses measurement of bone Gla-protein (BGP, also called Osteocalcin) and bone alkaline phosphatase (BAP, also called BALP as in the present application) in normal subjects and patients with metabolic bone disorders. Z scores were calculated. Both markers gave concordant results in patients with hypoparathyroidism, hyperthyroidism, primary hyperparathyroidism, acromegaly, and postmenopausal osteoporosis. This document recommends the use of both markers. See the analysis of Ref. (44) in D4, p.582, right col., last par. and p.585, left col., first par.

The subject-matter of claim 2 differs from this known method merely in that a marker that reflects the activity of osteoclasts is also determined. The aspect concerning metastases was not considered as it is not clear, see Item 2 above.

The problem to be solved by the present invention may therefore be regarded as how to evaluate the therapeutic effect of drugs used to treat hypoparathyroidism, hyperthyroidism, primary hyperparathyroidism, acromegaly or postmenopausal osteoporosis.

The solution proposed in claim 2 of the present application cannot be considered as involving an inventive step (Articles 52(1) and 56 EPC) because in view of D4 alone (see e.g. the abstract), the skilled person would regard it a normal design procedure to combine the determination of Osteocalcin and BALP as performed in Ref. (44) with a marker that reflects the activity of osteoclasts in order to solve the problem posed.

6. The Applicant's attention is drawn to the fact that the **requirements of Art. 82 EPC** must be met.
7. The Applicant is invited to amend the claims in a way that their wording is as close as possible to the wording of the claims as originally filed, taking into account the objections raised above.
8. To meet the requirements of Rule 27(1)(b) EPC, the **documents D1-D6 as well as the document cited under Ref. (44) in D4** should be identified in the description and



Beacheld/Protokoll (Anlage)

Communication/Minutes (Annex)

Notification/Procès-verbal (Annexe)

Datum
Date
Date

05.07.2007

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4

Anmelde-Nr.:
Application No.: 99 938 547.9
Demande n°:

the relevant background art disclosed therein should be briefly discussed.

9. The correction under Rule 88 EPC on p.9, l.6 can be accepted.
10. All objections listed above should be removed by suitable amendments in the next letter of reply. Should there still be substantial outstanding matter at that stage, **Oral Proceedings** as requested by the Applicant would have to be appointed.

The **description** should be adapted to any new set of claim filed in response to this communication.

Finally it is pointed out that all amendments must be carried out under strictest observation of **Article 123(2) EPC**.

The **time limit** has been set to **two (2) months** (Rule 84 EPC).



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23. Okt. 2007

Frist
bearb.:

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Application No. 99 938 547.9 - 2404	Ref. F 1079 EP S3	Date 22.10.2007
Applicant Ogata, Etsuro		

Communication under Rule 61(4) EPC

You are informed that the Examining Division intends to grant a European patent on the basis of the above application with the text and drawings as indicated below:

In the text for the Contracting States:

AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE

Description, Pages

1-17 filed with entry into the regional phase before the EPO
6a* introduced by the Examining Division

Claims, Numbers

1-6 received on 17.09.2007 with letter of 14.09.2007

Drawings, Sheets

1/4-4/4++ filed with entry into the regional phase before the EPO

With the following amendments to the above-mentioned documents by the examining division

Description, Pages 6*, 7**, 14**
Claims, Numbers 1+

Comments

* Rule 27(1)(b) EPC
** description adapted to the claims
+ Art. 84 EPC



Date 22.10.2007

Sheet 2

Application No.: 99 938 547.9

++ Drawing pages: version not corrected

A copy of relevant documents is enclosed

The title of the invention in the three official languages of the European Patent Office, the international patent classification, the designated Contracting States, the registered name of the applicant and the bibliographic data are shown on the attached EPO Form 2056.

You are requested within a **non-extendable** period of four months of notification of this communication

1.	to file 1 set of translations of the claim(s) in the two other EPO official languages;		EUR
2a.	to pay the fee for grant including the fee for printing up to and including 35 pages;		
	Reference 007		750.00
2b.	to pay the printing fee for the 36th and each additional page;		
	number of pages: 0		
		Reference 008	0.00
3.	to pay the additional claim fee(s) (Rule 51(7) EPC);		
	number of claims fees payable:		
		Reference 016	0.00
		Total amount	750.00

Concerning the possibility of a request for accelerated grant pursuant to Article 97(6) EPC, reference is made to OJ EPO 2001, 459.

If you do not approve the text intended for grant but wish to request amendments or corrections, the procedure described in Rule 51(5) EPC is to be followed.

If this communication is based upon an auxiliary request, and you reply within the time limit set that you maintain the main or a higher ranking request which is not allowable, the application will be refused (Article 97(1) EPC, see also Legal Advice 15/05 (rev. 02), OJ 6/2005, 357).

If the enclosed claims contain amendments proposed by the Examining Division, and you reply within the time limit set that you cannot accept these amendments, refusal of the application under Article 97(1) EPC would result in the case that agreement cannot be reached on the text for grant.

In all cases except those of the previous two paragraphs, if the grant, printing or claims fees are not paid, or the translations not filed, in due time, the European patent application will be deemed to be withdrawn (Rule 51(8) EPC).

For all payments you are requested to use EPO Form 1010 or to refer to the relevant reference number.

After publication, the European patent specification can be downloaded free of charge from the EPO publication server <https://publications.european-patent-office.org> or ordered only from the Vienna sub-office upon payment of a fee (OJ EPO 2005, 126).

Upon request in writing each proprietor will receive the certificate for the European patent **together with one copy** of the patent specification only if the request is filed within the time limit of Rule 51(4) EPC. If such request has been previously filed, it has to be confirmed within the time limit of Rule 51(4) EPC. The



Date 22.10.2007

Sheet 3

Application No.: 99 938 547.9

requested copy is free of charge. If the request is filed after expiry of the Rule 51(4) EPC time limit, the certificate will be delivered without a copy of the patent specification.

Translation of the priority document(s)

If the translation of the priority document(s), as required by Article 88(1) EPC, or the declaration according to Rule 38(5) EPC has not yet been filed, Form 2530 will be despatched separately. The translation is to be filed within the above mentioned time limit (Rule 38(5) EPC).

Note on payment of renewal fees

If a renewal fee falls due between notification of the present communication and the proposed date of publication of the mention of the grant of the European patent, publication will be effected only after the renewal fee and any additional fee have been paid (Rule 51(9) EPC).

Under Article 86(4) EPC, renewal fees are payable to the European Patent Office until the year in which the mention of the grant of the European patent is published.

Filing of translations in the Contracting States

Pursuant to Article 65(1) EPC the following Contracting States require a translation of the specification of the European patent in their/one of their official language(s) (Rule 51(10) EPC), insofar this specification will not be published in their/one of their official language(s)

- within **three** months of publication of the mention of such decision:

AT	AUSTRIA	FR	FRANCE
BE	BELGIUM	GB	UNITED KINGDOM
CH	SWITZERLAND / LIECHTENSTEIN	GR	GREECE
CY	CYPRUS	IT	ITALY
DE	GERMANY	NL	NETHERLANDS
DK	DENMARK	PT	PORTUGAL
ES	SPAIN	SE	SWEDEN
FI	FINLAND		

- within **six** months of publication of the mention of such decision:

IE IRELAND

The date on which the European Patent Bulletin publishes the mention of the grant of the European patent will be indicated in the decision on the grant of the European patent (EPO Form 2006).

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Bibliographical data of European patent application No. 99 938 547.9

For the intended grant of a European patent, the bibliographical data are set out below, for information:

Title of invention:

- VERFAHREN ZUR DIAGNOSE VON KNOCHENMETASTASEN AUS MALIGNEN TUMOREN
- METHOD OF DIAGNOSING BONE METASTASIS OF MALIGNANT TUMORS
- PROCEDE DE DIAGNOSTIC METASTASES OSSEUSES DE TUMEURS MALIGNES

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- **) In case two or more applicants have designated different Contracting States, this is indicated here.

~~METHOD OF DIAGNOSING BONE METASTASIS~~
~~OF MALIGNANT TUMORS~~

TECHNICAL FIELD

This invention relates to a method of diagnosing bone
5 metastasis accompanying malignant tumors such as breast
cancer, prostatic cancer and lung cancer; the invention
also relates to a method of evaluating the therapeutic
effect of drugs used to treat these diseases.

BACKGROUND ART

10 Bone metastasis of cancer is conventionally diagnosed
by examining clinical symptoms of the patient or images
taken by simple radiography, bone scintigraphy, CT, MRI,
etc. From a visual viewpoint, bone metastases are
classified as a dissolution type, a hardening type or a
15 mixed type depending on the balance between bone
dissolution and formation at the site of bone metastasis.
While image diagnoses are highly reliable and useful, they
are generally too expensive to be used for screening and
monitoring purposes.

20 With the recent advances in the study of bone
metabolism, various markers have been developed as indices
of bone metabolism. Markers of bone formation and
resorption are separately listed in Table 1. Attempts are
being made to diagnose bone metastases of cancers using
25 those bone metabolic markers (Koizumi, M. et al., Bone
Metabolic Markers in Bone Metastases, J. Cancer Res. and
Clin. Oncol., 121:541-548, 1995).

Table 1

Markers of bone formation

- (1) Type I procollagen peptides proliferation
 - C-terminal propeptide (PICP)
 - N-terminal propeptide (PINP)
- (2) Alkali phosphatases matrix formation
 - total alkali phosphatase (ALP)
 - bone alkali phosphatase (BALP)
- (3) Osteocalcin (OC) mineralization
 - C-terminal fragments
 - intermediate portions
 - intact

Markers of bone resorption

- (1) pyridinium cross-links
 - total urinary pyridinoline·deoxypyridinoline (HPLC method)
 - free urinary deoxypyridinoline (fDPD)
 - (2) pyridinium crosslinked collagen peptide fragments
 - serum C-terminal telopeptide (ICTP)
 - urinary C-terminal telopeptide (CTX)
 - urinary N-terminal telopeptide (NTx)
 - (3) Tartrate-resistant acid phosphatase (TRAP)
 - (4) Galactosyl hydroxylysine (GHYL)
 - (5) Hydroxyproline
 - (6) N-terminal osteocalcin
-

Most of the bone metabolic markers have as their rationale the measurement of metabolic products that are

released into blood and urine in the process of formation and absorption of type I collagen which accounts for 90% of the bone matrix. To be more specific, type I procollagen which is synthesized during bone formation releases C- and
5 N-terminal propeptides when it is converted to type I collagen and these propeptides serve as markers of bone formation. In the process of bone resorption, the type I collagen in the bone matrix undergoes metabolism to be released into blood and urine; the measured blood and urine
10 levels of the released type I collagen serve as markers of bone resorption.

Bone formation is known to consist of three major phases depending upon the stage of proliferation and differentiation of osteoblasts; the first phase is where
15 osteoblasts proliferate and the matrix forms, the second phase is for matrix maturation and the third phase is for calcification; and different markers are known to appear at different phases (Stein, G.S. et al.: Relationship of Cell Growth to the Regulation of Tissue-Specific Gene Expression
20 during Osteoblast Differentiation, FASEB J., 4:3111-3123, 1990).

In the phase of osteoblast proliferation and matrix formation, type I collagen forms actively and C- and N-terminal propeptides appear in the blood. In the phase of
25 matrix maturation, bone alkali phosphatase (BALP) is generated actively, causing BALP to be secreted into the blood. At the stage of calcification, osteocalcin (OC) appears. Bone formation is accelerated in OC-deficient

mice, suggesting that OC works as a suppressant of bone formation (Ducy, P. et al.: Increased Bone Formation in Osteocalcin-Deficient Mice; Nature, 382:448-452, 1996).

In the box of "Markers of bone formation" in Table 1,
5 "(1) proliferation" corresponds to the phase of osteoblast proliferation and matrix formation, "(2) matrix formation" to the phase of matrix maturation, and "(3) mineralization" to the phase of calcification.

While there are various markers of bone formation,
10 they frequently behave differently depending upon the state of the disease and it is important to realize specifically in which phase each marker appears.

There are also various markers of bone resorption and as with the markers of bone formation, metabolic products
15 of type I collagen are currently drawing special attention. In type I collagen, collagen of a triple-stranded structure occurs crosslinked with pyridinoline and deoxypyridinoline, so when it is destroyed upon bone resorption, pyridinoline and deoxypyridinoline cross-links having various sizes of
20 N- and C-terminal amino acids attached thereto are released into the blood.

The measurements of resorptive markers include that of cross-links alone (urinary pyridinoline and deoxypyridinoline that are measured as free entities), that
25 of cross-links including C-terminal amino acids (CTX and ICTP), and that of cross-links including N-terminal amino acids (NTx). For generalized details about bone metabolic markers, see the review article by Calvo et al. (Calvo,

M.S. et al., Molecular Basis and Clinical Application of Biological Markers of Bone Turnover, Endocrine Rev., 17:333-368, 1996).

In bone metastasis, markers of bone metabolism behave somewhat differently than in metabolic bone diseases such as osteoporosis. Among formative markers, increased PICP and BALP are observed in bone metastasis of prostatic cancer which is a typical example of bone hardening metastases but there is no significant increase in the level of osteocalcin which rises in osteoporosis and other metabolic bone diseases. The mechanism behind these phenomena is not presently known. In breast cancer which involves bone metastasis of a mixed type, the levels of formative markers increase but not as much as in prostatic cancer. In lung cancer which involves many cases of bone metastasis of a dissolution type, there are no significant increases in the levels of formative markers.

Among resorptive markers, ICTP differs from the other bone metabolic markers in that it does not change greatly after menopause but it has been found to increase in bone metastasis of cancer. From the viewpoint of detecting bone metastasis, ICTP may be considered a good marker that is insensitive to enhanced bone resorption in the post-menopausal stage. The levels of resorptive markers increase not only in bone metastasis of lung cancer which is mostly of a dissolution type but also in bone metastasis of breast cancer which is mostly of a mixed type, as well as in bone metastasis of prostatic cancer which is of a

hardening type.

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DISCLOSURE OF THE INVENTION

While the studies of bone metabolic markers have seen remarkable advances, comparisons of their advantages and limitations are presently far from being thorough and given many various markers of bone resorption and formation, one cannot tell for sure which markers are currently the best in diagnosis of bone metastasis.

In clinical diagnosis of bone metastasis, choice of a marker is entirely up to the discretion of each doctor and no technique has yet been established that allows for systematic monitoring of bone metastasis.

An object, therefore, of the present invention is to provide a tool capable of systematic monitoring of bone metastasis.

Under the circumstances, the present inventors conducted intensive studies with a view to developing a tool for systematic monitoring of bone metastasis and found that this object could be attained by combining a marker (formative marker) that reflects the activity of osteoblasts with a maker (resorptive marker) that reflects the action of osteoclasts. The present invention has been accomplished on the basis of this finding.

~~/Thus, according to one aspect of the invention/~~ There is provided a method of diagnosing bone metastasis of malignant tumors using a marker that reflects the activity of osteoblasts and a marker that reflects the action of osteoclasts.

→ p. 6a

6a

M. KOIZUMI: "Bone metastasis of cancer and osteal metabolism marker" CLINICAL CALCIUM, May 1998, pages 98 - 100, XP002922594, discloses a diagnostic method for bone metastasis of malignant tumors that uses a marker that reflects the activity of osteoblasts and a marker that reflects the activity of osteoclasts.

In TAKAHASHI S ET AL: "SIGNIFICANCE OF OSTEAL METABOLISM MARKER FOR DIAGNOSING BONE METASTASIS" BIOTHERAPY, KLUWER ACADEMIC PUBLISHERS, DORDRECHT, NL, vol. 11, no. 1, 1997, pages 75-80, XP002922593, ISSN: 0921-299X, combinations of markers are proposed.

K. NAKABA: "Correlation among the measurement of therapeutic effects on bone metastasis foci, osteal metabolism marker and the condition of bone metastasis" THERAPEUTIC RESEARCH vol. 16, no. 12, 1995, pages 212 - 217, XP002922592, describes means of determining therapeutic effect on bone metastasis lesions by using a marker that reflects osteoblast activity and a marker that reflects osteoclast activity.

In CHRISTENSON R H: "BIOCHEMICAL MARKERS OF BONE METABOLISM: AN OVERVIEW" CLINICAL BIOCHEMISTRY, PERGAMON PRESS, XX, vol. 30, no. 8, December 1997 (1997-12), pages 573-593, XP000938263, ISSN: 0009-9120, the combination osteocalcin + BALP is disclosed but used for another purpose.

PLEBANI M ET AL: "New and traditional serum markers of bone metabolism in the detection of skeletal metastases." CLINICAL BIOCHEMISTRY, vol. 29, no. 1, 1996, pages 67-72, XP002184395, ISSN: 0009-9120, have tested several markers but osteocalcin was not found to be useful.

MEIJER W G ET AL: "Biochemical parameters of bone metabolism in bone metastases of solid tumors (Review)". ONCOLOGY REPORTS, Vol. 5, No. 1, January 1998, pages 5-21, XP001027215, ISSN: 1021-335X, do not disclose any specific combination of markers.

DUDA R J JR ET AL: "Concurrent assays of circulating bone Gla-protein and bone alkaline phosphatase: effects of sex, age, and metabolic bone disease" THE JOURNAL OF CLINICAL ENDOCRINOLOGY AND METABOLISM, vol. 66, no. 5, May 1988 (1988-05), pages 951-957, XP009089843, United States, disclose measurement of bone Gla-protein (BGP, also called Osteocalcin) and bone alkaline phosphatase (BAP, also called BALP as in the present application) in normal subjects and patients with metabolic bone disorders. Z scores were calculated. Both markers gave concordant results in patients with hypoparathyroidism, hyperthyroidism, primary hyperparathyroidism, acromegaly, and postmenopausal osteoporosis. Discordant results, however, were observed with osteolytic metastasis and other conditions.

→ 7

~~/According to another aspect of the invention/~~ There
^{also} is provided a method of evaluating the therapeutic effect
of drugs using a marker that reflects the activity of
osteoblasts and a marker that reflects the action of
osteoclasts. The invention is defined by the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a diagram showing how three different
markers begin to appear as osteoblasts differentiate;

Fig. 2 is a graph showing the relationship between
the efficacy of chemotherapy on patients with prostatic
cancer involving bone metastasis and each of three markers,
PICP, BALP and osteocalcin;

Fig. 3 is a graph showing the changes in a crossover
index, osteocalcin/BALP, for patients with breast cancer;
and

Fig. 4 is a graph showing the changes in the ICTP
level during treatment of breast cancer.

BEST MODE FOR CARRYING OUT THE INVENTION

We now describe the findings which were the basis for
accomplishing the present invention.

(1) Markers of bone formation as produced from osteoblasts:

As Fig. 1 shows, the differentiation of osteoblasts
involves shifts in marker expression from PICP and PINP
(type I procollagen peptides) through BALP (bone alkali
phosphatase) to osteocalcin (Stein, G.S. et al., FASEB J.,
4:3111-3123, 1990).

(2) Reactions of BALP and osteocalcin to chemotherapy in
patients with prostatic cancer and breast cancer who were

suffering from bone metastasis:

The levels of two formative markers, BALP and osteocalcin, differed with the state of bone metastasis, as demonstrated in the following two Examples.

5 Example 1

During the period from October 1994 to April 1996, the levels of formative markers were measured in 43 prostatic cancer patients with bone metastasis and 46 prostatic cancer patients without bone metastasis. Of the 10 46 prostatic cancer patients who apparently had no bone metastasis, 29 had received prostatectomy or radiation therapy and the remaining 17 were newly diagnosed patients who received prostatectomy or radiation therapy after bone scintigraphy and serum sampling. The patients without bone 15 metastasis were aged 69 on average (ranging from 47 to 85 years old). The progress of prostatic cancer in these patients was as follows: four patients at stage A, 14 at stage B, 19 at stage C, and 9 at stage D1. Of the 43 patients with bone metastasis, 9 were newly diagnosed and 20 received hormone therapy after bone scintigraphy and serum sampling. The remaining 34 patients had received positive treatments by hormone therapy and/or chemotherapy at various time intervals from the start of these treatments. The patients with bone metastasis were aged 69 on average 25 (ranging from 53 to 83 years old).

After obtaining informed consent from all patients, blood samples were taken during bone scintigraphy and sera were separated and stored frozen at -40°C until analysis.

Serum BALP was measured by enzyme immunoassay with an ALKPHASE-B kit of Metra Biosystems. Serum osteocalcin was measured by immunoradiometric assay with a BGP-IRMA kit of Mitsubishi Chemical Corp.

5 The results are shown in Fig. 2, in which Z value is defined by (measured value - average for the patients without bone metastasis)/(standard deviation of a patient without bone metastasis). In Fig. 2, CR, flare, NC, IMP, new and PD have the following respective meanings.

10 CR : complete remission

flare : flare-up [the treatment was effective but the bone metastasis appeared to have progressed on a bone scan (scintigraphic) image].

NC : no change (no change was observed).

15 IMP : improvement (a sign of improvement was recognized).

new : diagnosed to have a new bone metastasis.

PD : progression of disease (the disease was found to have progressed).

20 For each of these patient groups, BALP and osteocalcin had the following Z values.

Z value of BALP

CR : 2.18

flare : 3.40

NC : 8.23

25 IMP : 2.39

new : 1.82

PD : 24.50

Z value of osteocalcin

CR : 1.30
flare : -0.18
NC : 0.04
IMP : 1.25
5 new : 0.08
PD : 0.05

Using these values, a crossover index (Z
osteocalcin/Z BALP) was calculated for each patient group.

Crossover index

10 CR : 0.596
flare : -0.053
NC : 0.005
IMP : 0.523
new : 0.044
15 PD : 0.002

As is clear from the above data, BALP had a low Z
value (2.18) in the CR group in which the treatments proved
effective whereas it had a high Z value (24.50) in the PD
group in which the disease worsened. On the other hand,
20 osteocalcin had a high Z value (1.30) in the CR group but
had a low Z value (0.05) in the PD group. The crossover
index was 0.596 in the CR group but 0.002 in the PD group,
with a marked difference being observed between the two
groups. It can hence be concluded that the crossover index
25 allows for both diagnosis of the progression of bone
metastasis and evaluation of drug efficacy in the treatment
of the disease.

Example 2

As in Example 1, the levels of formative markers (BALP and osteocalcin) were measured in a total of 850 patients with breast cancer, 644 of whom had bone metastasis and 206 having no bone metastasis. The patients with bone metastasis received chemotherapy or endocrine therapy targeted to the site of bone metastasis; they were classified into six groups, CR, NC, IMP, new and PD, according to the therapeutic efficacy achieved and the Z values of BALP and osteocalcin were determined in each group. On the basis of the measured Z values, a crossover index was calculated for each patient group.

After obtaining informed consent from all patients, blood samples were taken during bone scintigraphy and sera were separated and stored frozen at -40°C until measurement. Serum BALP was measured by enzyme immunoassay with an ALKPHASE-B kit of Metra Biosystems. Serum osteocalcin was measured by immunoradiometric assay with a BGP-IRMA kit of Mitsubishi Chemical Corp. The results are shown below.

Z value of BALP

CR	: 0.741
NC	: 1.514
IMP	: 0.735
new	: 2.021
PD	: 5.041

Z value of osteocalcin

CR	: 0.267
NC	: 0.237

IMP : 0.039

new : -0.167

PD : 0.516

Crossover index

5 CR : 0.360

NC : 0.157

IMP : 0.053

new : -0.083

PD : 0.102

10 Fig. 3 is a graph showing the changes in crossover
index as observed in the respective groups CR, NC, IMP, new
and PD. Obviously, the crossover index for the group CR in
which the treatments proved effective was 0.360 whereas the
value for the group PD in which the disease worsened was
15 0.102, with a marked difference being observed between the
two groups. It can hence be concluded that as in the case
of prostatic cancer, the crossover index allows for both
diagnosis of the progression of bone metastasis of breast
cancer and evaluation of drug efficacy in the treatment of
20 the disease.

The results of Examples 1 and 2 showed that the
patients with bone metastasis in group CR who were
effectively treated by drugs had high crossover index
values whereas the patients with bone metastasis in group
25 PD who changed for the worse without any therapeutic effect
had low crossover index values. It was therefore supported
that a crossover index between two osteoblast markers was
extremely effective in evaluating the degree of amelioration

of bone metastasis (therapeutic efficacy of drugs).

In patients with prostatic cancer, the crossover index value of group CR was close to that of group IMP and so was the crossover index value of group NC to that of group PD; these data reflect the therapeutic efficacy for bone metastasis of prostatic cancer which was predominantly attributable to bone formation. The result from the patients with breast cancer was somewhat different in that the crossover index value of group NC was close to that of group PD whereas the crossover index value of group CR was not close to that of group IMP. The difference would have originated because breast cancer presents an image of bone metastasis to which bone dissolution is a more significant predisposing factor than bone formation. Therefore, in order to ensure that the progress of bone metastasis (the degree of aggravation) is diagnosed accurately, not only formative markers but also resorptive markers would have to be measured.

In Examples 1 and 2, the progress of bone metastasis of malignant tumors and the efficacy of their treatment by drugs were diagnosed by measuring the crossover index between osteocalcin which is a marker associated with the phase of calcification and BALP which is a marker associated with the phase of matrix maturation. The present inventors also verified that the progress of bone metastasis of malignant tumors and the efficacy of their treatment by drugs could be diagnosed by measuring the crossover index between osteocalcin which is a marker

associated with the phase of calcification and PICP and
PINP which are a marker associated with the phase of
osteoblast proliferation and matrix formation. ~~Needless to~~
say, osteocalcin can be replaced by any other markers that
5 are associated with the phase of calcification, PICP, or
PINP can be replaced by any other markers that are
associated with the phase of osteoblast proliferation and
matrix formation, and BALP can be replaced by any other
~~markers that are associated with the phase of matrix~~

10 ~~maturation~~

In the past, several markers of bone formation have
been identified and their levels have been individually
measured to show that different markers were produced at
different times depending on the stage in differentiation
15 and maturation of osteoblasts. However, it has not been
shown clearly which of the formative markers should be
exclusively used as indices of bone metastasis to reflect
the fact that "differentiation and maturation of
osteoblasts are suppressed by bone metastasis of cancer".

20 It was entirely unexpected from the prior art that the
progress of bone metastasis and the efficacy of its
treatment by drugs could be evaluated by the above-defined
crossover index.

The bone to which cancer has metastasized is broken
25 down by osteoclasts. While several markers are known to be
capable of evaluating the bone resorption that accompanies
bone destruction, the degree of bone metastasis (worsening
of the disease) and the effect of therapy in suppressing

bone destruction can be evaluated definitely by identifying ICTP (type I collagen carboxy-terminal telopeptide) which is a collagen metabolite having a comparatively high molecular weight, validating ICTP as a reliable marker of bone resorption (see, for example, The Bone, Vol. 10, No. 3, pp. 111-118, 1996). Described below is an example showing the degree of bone metastasis and the effect of therapy in suppressing bone destruction.

Example 3: ICTP Level in Treatment of Breast Cancer

10 ICTP levels were measured in 23 patients with breast cancer who had received chemotherapy of bone metastasis by CAF regimen (C, cyclophosphamide; A, doxorubicin or Adriamycin; F, fluorouracil). The control group consisted of 9 patients with breast cancer who had no bone metastasis and received a CAF regimen as adjuvant therapy.

15 After obtaining informed consent from all patients, bone metabolic markers indicative of bone formation and resorption were measured. At the onset of CAF treatment and up to its end, blood samples were taken when bone scintigraphy was performed once a month and the sera were separated. The separated sera were stored frozen at -40°C until analysis. A formative marker BALP was measured by enzyme immunoassay with an ALKPHASE-B kit of Metra Biosystems. A resorptive marker ICTP was measured by radiimmunoassay with Orion Diagnostica. The serum CA 15-3 was measured by immunoradiometric assay with Centocor. The measured values were expressed in terms of the average and SE (standard error). A test of significance was carried

out by analysis of variance (ANOVA) according to the Bonferroni method.

The results are shown in Fig. 4, from which one can see that the ICTP values in the patients of group PD increased significantly over the ICTP values in the patients of group PR (partial response) and NC. The ICTP values of the patients of the "flare" group were significantly lower than those of the patients of group PD and substantially the same as those of the groups NC and PR without flare-up. The terms PD, flare and NC in Fig. 4 have the same meanings as in Example 1. PR means "partial therapeutic effect recognized".

No statistically significant difference was shown by the values of BALP and CA 15-3.

It is therefore concluded that by measuring the ICTP level, one can evaluate the degree of exacerbation of cancer metastasis to bone.

According to the findings in Examples 1 - 3, the amelioration of bone metastasis (therapeutic effect) and the degree of its exacerbation can be correctly diagnosed by monitoring two markers, one associated with osteoblasts and targeted to evaluation of therapeutic effect and the other associated with osteoclasts and targeted to evaluation of the worsening of the disease.

25 INDUSTRIAL APPLICABILITY

As described on the foregoing pages, the present invention provides a tool by which bone metastases caused by malignant tumors such as breast cancer, prostatic cancer

and lung cancer and the therapeutic efficacy of drugs for the cancers causative of such metastases can be diagnosed much more accurately than by the prior art methods.

EP 99 93 8547.9
Etsuro Ogata
Our Ref.: F1079 EP S3

CLAIMS

1. An in vitro method for diagnosing amelioration and/or exacerbation of bone metastasis accompanying malignant tumors in a patient with a cancer disease using ~~a~~ marker that reflects the activity of osteoblasts and a marker that reflects the activity of osteoclasts, wherein the marker that reflects the activity of osteoblasts ~~is~~ osteocalcin in combination with BALP or PICP or PINP. *are*
2. The method of claim 1, wherein diagnosing amelioration and/or exacerbation of bone metastasis accompanying malignant tumors in a patient with a cancer disease involves evaluating the therapeutic efficacy of a drug.
3. The method of claim 2, wherein the drug is a cancer control therapeutic agent, a bone resorption suppressant, or an endocrine therapeutic agent.
4. The method of claim 1 to 3, which is based on the value of a crossover index or the ratio between osteocalcin and BALP or PICP or PINP, and the measured value of the marker that reflects the activity of osteoclasts.
5. The method of any one of claims 1 to 4, wherein the marker that reflects the activity of osteoclasts is a marker associated with bone type I collagen.
6. The method of any one of claims 1 to 5, wherein the marker that reflects the activity of osteoclasts is deoxypyridinoline and/or ICTP.

Fig. 1

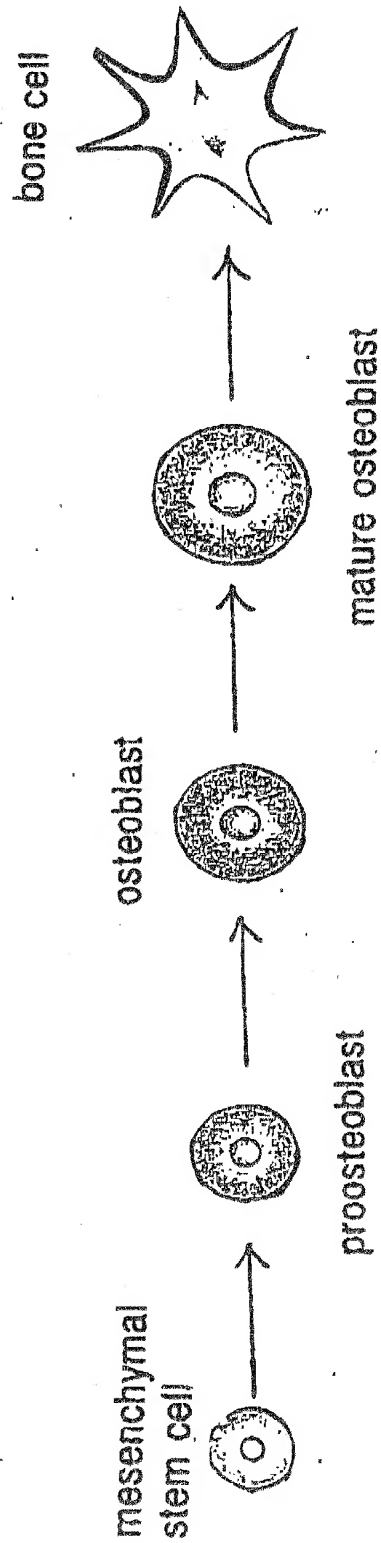
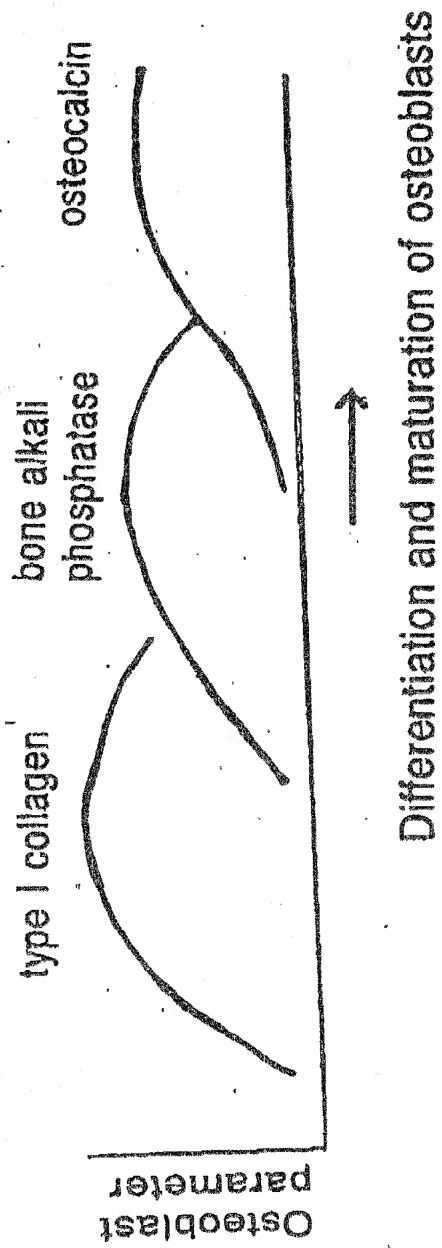


Fig. 2

Relationship between Treatment of Prostatic Cancer Patients
and Markers of Bone Formation

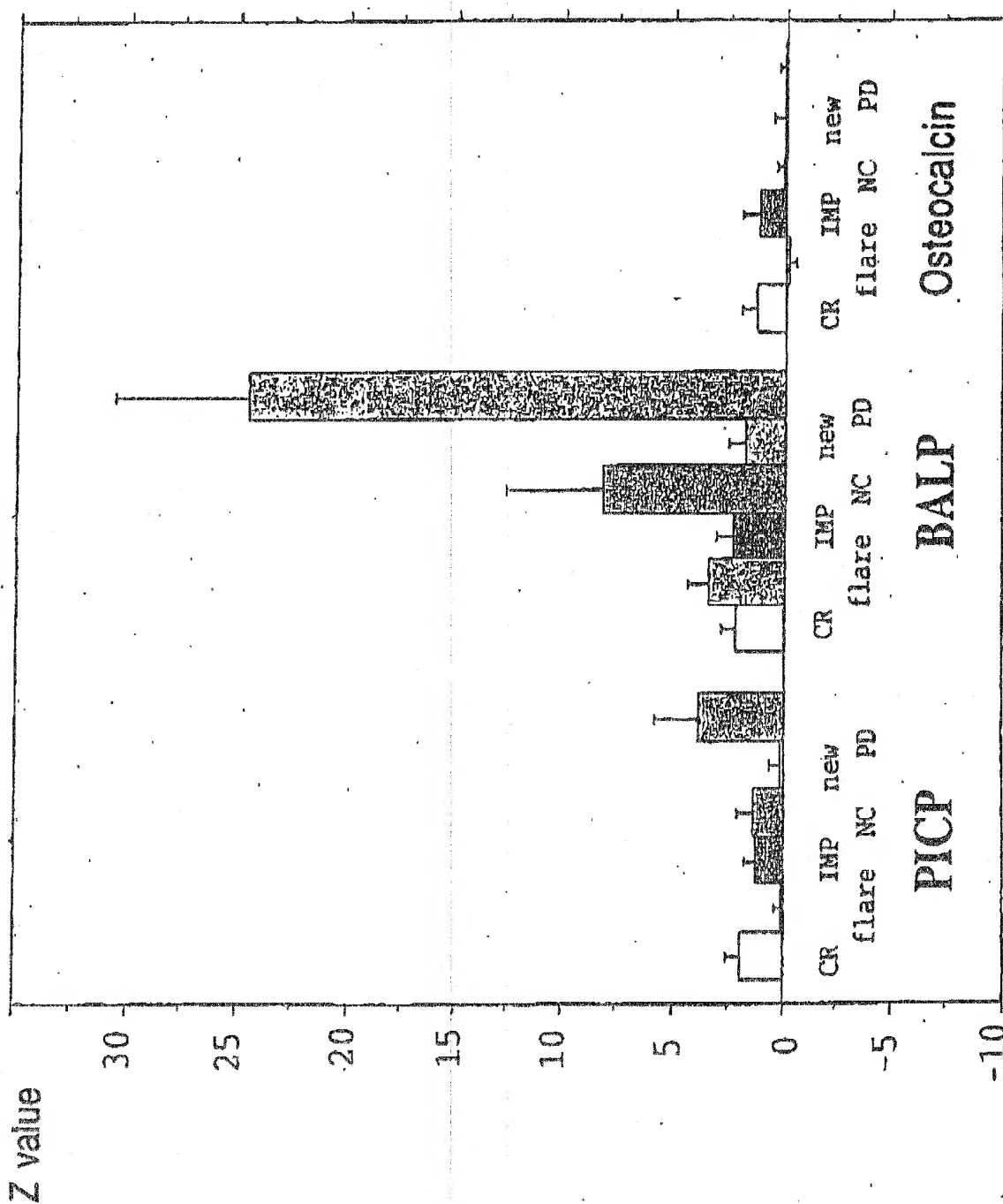


Fig. 3

Changes in Crossover Index after Chemotherapy
(in Cases of Bone Metastasis of Breast Cancer)

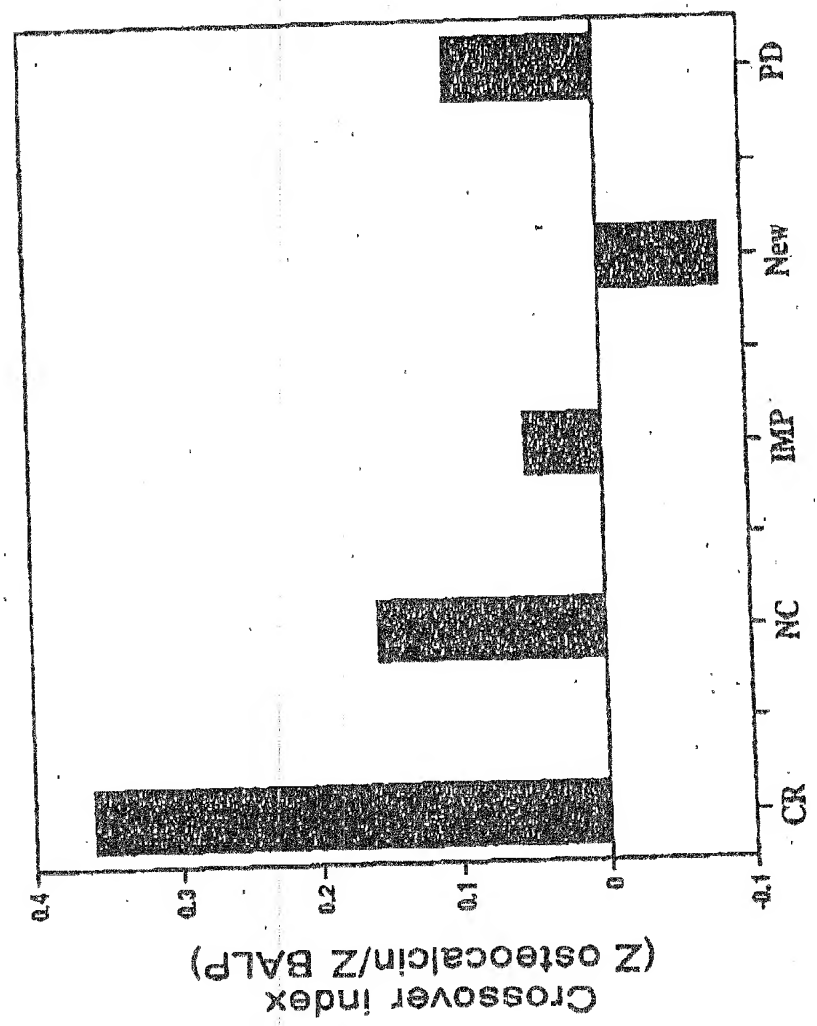


Fig. 4

Changes in ICTP Level during Chemotherapy by CAF Regimen

(in Patients with Breast Cancer)

